19. MORPHOLOGICAL DIFFERENTIATION OF THE LARVAL INSTARS OF *SIMULIUM ORNATUM* MEIGEN (NEMATOCERA, DIPTERA), WITH A NOTE ON ITS METAMORPHOSIS AND ECOLOGY

(With a plate)

The following observations were made on thousands of living specimens in the streams of Priddy and Limpleystoke, Bristol, England, and also on larvae kept alive in large aquaria in the zoology laboratory of the University of Bristol at 14°-16° C., and artificially aerating the water.

It is a very old idea to presume that larvae are precocious embryos. According to Lubbock (1874), the occurrence of metamorphosis arises from the immaturity of the condition in which some animals quit the egg. This may be true in the case of the primary larvae of some parasitic Hymenoptera, which have unsegmented abdomens and undeveloped respiratory and nervous systems on hatching. This is not true, however, of all insect larvae, particularly of the *Simulium* larva, because its morphological features and anatomical structures are very well developed, even at the time of emergence from the egg.

The larva of *Simulium ornatum* lives attached to stones or weeds in swift-flowing streams (5-7 ft. = 1.5-2.1 m. per second). Some of the outstanding features possessed by the larva to cope with its aquatic environment are: the specialized mouth brush for collecting food; the sticky salivary secretion, often used for suspension when it is detached; the thoracic proleg and the posterior sucker with hooks, enabling movement and fixation; and anal gills for respiration.

When the larva is detached from its point of attachment, it clings on to a silken thread (salivary secretion) and soon regains its original position. In this endeavour the thoracic proleg and the posterior sucker are of immense help. The posterior sucker is the main organ of attachment. The radiating rows of hooks, about seventy-five in number, strongly grip the sticky salivary secretion which fills up the spaces between the hooks, forming a complete rim all round. The term 'posterior sucker', adopted by many authors, suggests the idea that this organ functions like a true sucker. Helped by the muscles attached to the periphery of the sucker and the centre of the disc, this organ actually works like a sucker, especially at the initial stage of attachment, when the salivary secretion is not sticky enough for the firm fixation of the hooks.

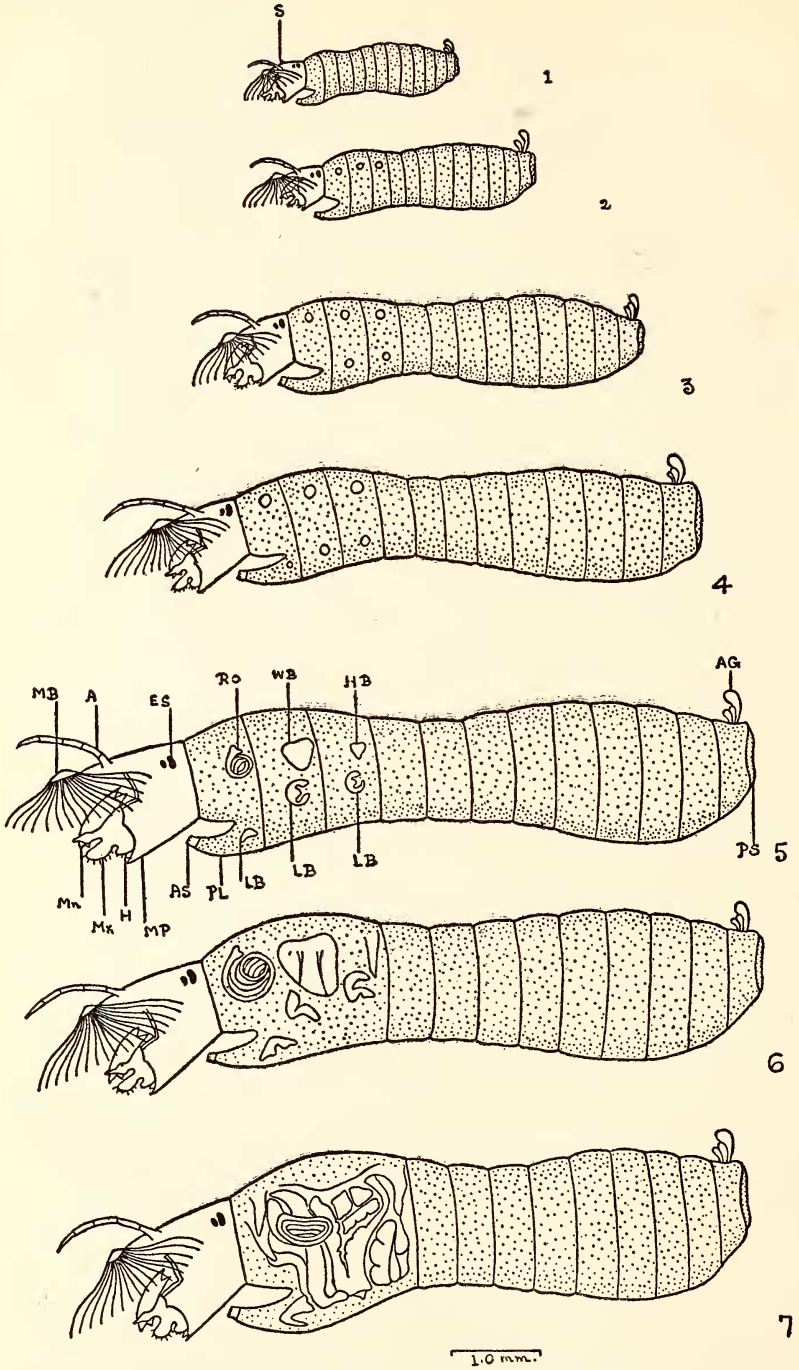

Fig. 1. 72-hour old first instar larva ; Fig 2. 120-hour old second instar larva ; Fig. 3. 96-hour old third instar larva ; Fig. 4. 72-hour old fourth instar larva ; Fig. 5. 120-hour old fifth instar larva ; Fig. 6. 72-hour old sixth instar larva ; Fig. 7. 108-hour old pharate pupa.

A. Antenna, AG. Anal gill, AS. Anterior sucker, ES. Eye spot, H. Hypopharynx, HB. Haltere bud, LB. Leg bud, MB. Mouth brush, MN. Mandible, MP. Mental plate, Mx. Maxilla, PL. Proleg, PS. Posterior sucker, RO. Respiratory organ, S. Spine, WB. Wing bud.

Usually three generations are produced every year, with longer intervals between the various stadia, during the winter.

According to Puri (1925) and Smart (1944) there are three thoracic and eight abdominal segments in *Simulium* larvae. But, actually there are three thoracic and nine abdominal segments, as suggested by Grunberg (1910) and Hermes (1923). This is clearly revealed by the arrangement of the muscles in the larva of *Simulium ornatum* (to be published elsewhere).

During the initial stages of an instar, when the old and new cuticles lie near to each other, the space between them is filled by the exuvial fluid. Later on, when the space between them increases, the moulting fluid is withdrawn and the space is partially filled with air. At this stage the outer cuticle lies around the body as a loose bag and many tonofibrillae connections between the two cuticles become gradually detached. The old cuticle usually stays on for several hours, even after all the tonofibrillae connections between the two cuticles have become completely detached. This often leads to underestimation of the age of the larva. Generally in calculating the duration of each instar, the time spent by the new instar within the cuticle of the previous instar is attributed to the old instar. But, actually the moult has occurred already, though ecdysis has not taken place. As pointed out by Hinton (1958), the detachment and the retraction of the epidermis from the cuticle indicates the real moult.

First instar larva (Fig. 1). The three thoracic and nine abdominal segments, with all the specialized organs are present in this instar. A spine, the egg burster, situated dorso-medianly on the head in between the eyes, is a characteristic organ of this larval instar. It helps the larva to come out of the egg. The imaginal buds are not morphologically evident. The length of the body is about 2 mm. and thickness about 0.5 mm. This instar lasts for five to seven days.

Second instar larva (Fig. 2). The body is cylindrical in shape. Three pairs of small spherical imaginal buds, one pair in each of the thoracic segments, situated dorso-laterally, become morphologically visible. The imaginal buds are not very distinct and conspicuous.

The imaginal bud in the first thoracic segment is destined to give rise to the respiratory filament, that in the second thoracic segment to the wing, and that in the third thoracic segment to the haltere. The body is about 3 mm. long and 0.8 mm. thick. This larval instar lasts from six to eight days.

Third instar larva (Fig. 3). During this stage the contour of the body becomes a little altered. The thorax becomes humped and the abdomen gradually assumes the clubbed shape. Abdominal segments one to three are comparatively smaller than the rest. In addition to the three thoracic imaginal buds of the previous instar, two imaginal buds become evident, situated ventro-laterally in the second and third thoracic segments. These two buds are destined to develop into legs. The length of the larva is about 5 mm. and the thickness is about 1 mm. This larval instar lasts for seven to nine days.

Fourth instar larva (Fig. 4). Another pair of buds appear on the ventro-lateral aspect of the prothorax, near the place where the proleg joins it. This is destined to develop into the prothoracic leg. This is the six-bud stage. No more buds appear, but these six buds gradually expand in size due to the multiplication of cells. The length of the body is about 7.5 mm. and the thickness is about 1.2 mm. This larval instar lasts for six to eight days.

Fifth instar larva (Fig. 5). The larva attains its maximum length at this stage. The humped thorax, club-shaped abdomen, and cylindrical body gives the larva its characteristic graceful contour. The respiratory bud becomes black in colour and shows the developing respiratory organ inside. The wing and the haltere buds have become roughly triangular in shape, with the base of the triangle towards the dorsal aspect of the body. The wing bud is larger than the haltere bud. The leg buds of the meso- and meta-thoracic segments show the developing legs inside. The prothoracic leg bud remains smaller than the other two leg buds. The length of the body is about 9 mm. and the thickness is about 1.5 mm. This larval instar lasts for six to eight days.

Sixth instar larva (Fig. 6). Due to rapid segmentation, all the buds become enlarged and show the characteristic shape of the organ into which they are destined to develop. The superficial lines of demarcation between the three thoracic segments disappear. But the three thoracic segments can be differentiated with the help of their developing imaginal organs. The length of the body is about 9 mm. and the thickness about 1.8 mm. This larval instar lasts for six to eight days.

Pharate pupa (Pre-pupa) (Fig. 7). This is actually the pupa within the larval cuticle. According to Hinton (1946), the term 'pharate' denotes the phase of an instar which is enclosed within the cuticle